

Two *Escherichia coli* Chromosomal Cistrons, *sfrA* and *sfrB*, Which Are Needed for Expression of F Factor *tra* Functions

LOTHAR BEUTIN† AND MARK ACHTMAN*

Max-Planck-Institut für molekulare Genetik, D-1000 Berlin 33, Germany

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Twelve mutants of *Escherichia coli* K-12 have been isolated which carry chromosomal mutations that exhibit pleiotropic effects on the expression of F factor *tra* cistrons. F pilus synthesis, deoxyribonucleic acid transfer, and surface exclusion are all inhibited. Six of the mutants carry *sfrA* mutations, and six carry *sfrB* mutations. *sfrA* and *sfrB* are cistrons mapping near *thr* and *metE*, respectively. Several F-like plasmids are dependent on *sfrA* and on *sfrB* for expression of *tra* cistrons. Plasmids of incompatibility groups C and S are only dependent on *sfrB*, and other conjugative plasmids are dependent on neither. *sfrB* mutations also result in changes in certain cell envelope properties, including changed sensitivity to certain bacteriophages which use lipopolysaccharide as a receptor, synthesis of nonfunctional flagella, and altered sensitivity to antibiotics.

Escherichia coli cells carrying the F sex factor act as good donors and as poor recipients in conjugation. Numerous F-encoded *tra* cistrons (for review, see ref. 3) have been identified which are responsible for F pilus synthesis, mating aggregation, and DNA transfer; others have been identified which are responsible for poor recipient ability (here called surface exclusion). Most of these cistrons are located in the *tra* operon (17), the transcription of which requires expression of the *traJ* gene (14, 29). *traJ* expression can be prevented by the *finO* and *finP* gene products (12, 13, 29). The F factor is *finO* and thus shows constitutive expression of the *tra* operon. When a second plasmid capable of supplying the *finO* protein is introduced into cells carrying an F factor, *traJ* transcription and *tra* operon transcription are inhibited (29).

In addition, expression of the *tra* cistrons of the F plasmid depends on the functional integrity of the transcription and translation machinery of the cell (10, 24-26) and presumably on other important aspects of cell function. However, it was not clear whether certain cell components played a specific role in the expression and function of the F factor cistrons. Here we describe the isolation of *E. coli* K-12 mutants with properties suggesting that the host cell does play such a specific role.

(These results have been presented in partial fulfillment of the requirements for a doctorate [L.B., Inaugural-Dissertation, Freien Universität, Berlin, 1978].)

† Present address: Bundesgesundheitsamt, Max von Pettenkofer Institut, D-1000 Berlin 33, Germany.

MATERIALS AND METHODS

Those bacterial strains critical to this study were *E. coli* K-12 derivatives (Table 1). Hfr strains used for linkage analysis were obtained from the *E. coli* Genetic Stock Center. RecA derivatives were obtained after conjugation with the Hfr donor strain JC10240 and selection for tetracycline resistance. Of the Tet^r transconjugants, 90% were RecA⁺, as shown by sensitivity to UV light and recombination deficiency after conjugation. The sex factors used were from various sources: *Flac* (JCFLO [5]), *Fgal* (F8 [18]), *Ftrp* (from R. Thompson; original source unknown), and R100 *finO* (UCR105 [19]) were from the laboratory strain collection; R1drd-19 was obtained from K. N. Timmis; ColV2, ColVBtrp, and R538-10⁺ were obtained from N. Willetts; R₁₀₀-1 was obtained from Y. Terawaki; and the remainder listed in Table 4 were from N. Datta. The original sources of these latter plasmids have been cited by Jacob et al. (20). Bacteriophages M12, f2, ϕ_{11} , and P1 were from the laboratory bacteriophage collection; χ was obtained from B. Lugtenberg; the remainder were obtained from P. Reeves via P. A. Manning. Bacteriophages were propagated on *E. coli* K-12 strain P400, with the exception of bacteriophages T1, T2, T3, T4, T6, T7, and C21, which were grown on *E. coli* B. Media and techniques used for growing these bacterial strains and bacteriophages have been described or cited elsewhere (1, 2, 5, 6, 30). Bacteriophage χ was propagated on strain PC1349 from B. Lugtenberg, using the techniques and media described by Schade and Adler (27). Bacterial swarming was tested as described by Armstrong et al. (7).

Curing with acridine orange. Plates containing acridine orange were made as described by Fan (11), except that the pH of the medium was adjusted to 8.5 before autoclaving, and acridine orange (E. Merck AG, Darmstadt, Germany) was present at a concentration of 75 μ g/ml. Bacterial suspensions were streaked to single colonies on the plates, and the plates were

TABLE 1. *Bacterial strains*

Strain	Relevant properties	Source/reference
AB2463	Thr ⁻ Leu ⁻ Ara ⁻ RecA ⁻ , carries KLF1	CGSC4250 ^a
C600	Thr ⁻ Leu ⁻ LacY ⁻ Str ^r SuII ⁺	A. Reiner
FOS-3	Ilv ⁻ Cya _Δ ⁻	M. Malamy
IO7011	Trp ⁻ His ⁻ Ilv ⁻ MetE ⁻	J. Beckwith
JC1553	RecA ⁻ ; carries KLF33	CGSC4265 ^a
JC3272	F ⁻ B ₁ ⁻ His ⁻ Trp ⁻ Lys ⁻ Gal ⁻ MalA ⁻ Str ^r T6 ^r Su ⁻	5
JC10240	Hfr <i>srl-300::Tn10 recA56</i>	A. J. Clark
M176	As JC3272 but carries <i>Flac</i>	5
M1986	LacY ⁻ Thr ⁻ Ara ⁻ ThyA ⁻ SuII ⁺ Str ^r , carries <i>Flac</i>	Leu ⁺ Ara ⁻ Thy ⁻ C600 ^b
M3960	Trp ⁻ His ⁻ MetE ⁻ Cya _Δ ⁻ , carries <i>Ftrp</i>	Ilv ⁺ Cya _Δ ⁻ IO7011 ^c
P400	Sensitive to most bacteriophages	P. Reeves

^a *E. coli* Genetic Stock Center; obtained from B. Bachmann.

^b The *thyA* mutation was selected by treatment with trimethoprim.

^c FOS-3 was transduced to Ilv⁺ with P1 bacteriophage. A P1 lysate made on this strain was used to transduce IO7011 to Ilv⁺ Cya⁻.

incubated in the dark at 37°C for 2 to 3 days. The use of lactose or galactose as a fermentable sugar allowed the ready recognition of colonies or colony sectors which had been cured of *Flac* or *Fgal*, respectively. Most colonies contained only cured cells or exhibited numerous cured sectors. No curing on these plates has been observed for any of the F-like R factors which have been tested.

Mutagenesis. M176 was grown in L broth to a density of 4×10^8 cells per ml, and the cells were centrifuged and suspended in one-half volume of minimal medium 56/2, supplemented with histidine, tryptophan, lysine, and vitamin B₁ but without any carbon source. Ethyl methane sulfonate (Pierce Chemical Corp., Rockford, Ill.) was diluted 50-fold in 5 ml of supplemented minimal medium (as above) and incubated at 37°C for 30 min to allow settling of undissolved droplets. Then 0.1 ml of the bacterial suspension was layered on the mixture, and incubation continued for a further 30 min. A 0.1-ml amount of the mutagenized bacteria was diluted in 10 ml of L broth and incubated overnight at 37°C to allow segregation and expression of mutants.

Assays for the Sfr⁻ phenotype and for measuring DNA transfer. Colonies of cells carrying an F-prime sex factor are sensitive to male-specific bacteriophages M12 and f2 unless they harbor mutations preventing F pilus synthesis. *sfr* mutations do prevent F pilus synthesis and can be readily detected by screening colonies by replica plate techniques for resistance to male-specific bacteriophages (4). With these techniques, 1 in 1,000 colonies could readily be recognized which differed from the remainder in the Sfr phenotype. On occasion, replica plate techniques were used to screen for transfer deficiency, a second aspect of the Sfr⁻ phenotype. Quantitative analyses of plasmid DNA transfer efficiency were performed by measuring the ability to transfer a sex factor in 40-min matings at 37°C as described (4).

Surface exclusion with recipient cells carrying an F prime was measured by using the Hfr donor M2311 and a slightly different technique, necessitated by the observation that DNA transfer and/or recombination is inefficient at high cell densities with Hfr donors (1). These mating mixtures each contained 1 ml of an Hfr culture grown in L broth at 37°C to 2×10^8 to 4×10^8 cells per ml without agitation and 1 ml of the test culture grown similarly but with agitation. After 20 min at 37°C without agitation, the mating mixtures were diluted 100-fold in warm L broth, and 60 min of incubation (without agitation) was allowed for transfer and recombination. His⁺ [Str^r] recombinants were selected, and the surface exclusion index was calculated relative to an *sfr⁻* F⁻ strain. The same mating protocol was used with Hfr \times F⁻ mating mixtures for mapping *sfr* mutations by linkage analysis, except that 2 h of incubation at 37°C was allowed after dilution when the calculated time of entry of the selected allele was more than 20 min.

Electron microscopy. M12 bacteriophages were used to label F pili, or χ bacteriophages were used to label functional flagella. A total of 10^{10} bacteriophages was added to 1 ml containing 2×10^8 bacteria. In both cases, formaldehyde (final concentration, 2.5%) was used (at 0°C) to fix exponentially growing cells before samples were applied to grids for electron microscopy. For F pilus analysis, formaldehyde and M12 bacteriophages were added simultaneously, whereas for flagella analysis, χ bacteriophages were added 10 min before the formaldehyde. Samples were applied to carbon- or Formvar-coated grids immediately after formaldehyde addition or just after overnight fixation. Negative staining was performed with 2% phosphotungstic acid (pH 7.7) or 1.5% uranylacetate (pH 4.0). The grids were evaluated with a Philips EM301 transmission electron microscope.

RESULTS

Isolation of Sfr⁻ mutants. The procedure described below resulted in the isolation of mutants which prevented the normal function of F factor *tra* operon cistrons. Our original hope was to isolate *finO*⁺ revertants of the F sex factor (which is a naturally occurring *finO* plasmid), and the mutant isolation was designed accordingly (Fig. 1). First we used conjugation with an Hfr donor to enrich (40-fold or more) for mutants of a strain carrying *Flac* which had become good recipients (deficient in surface exclusion). Then the cells were screened for the inability to synthesize F pili even when an unmutagenized *finO* R plasmid was introduced. Note that F pilus synthesis and surface exclusion are encoded by independent *tra* operon cistrons (3). Several additional features were included in the protocol to maximize success.

(i) Sodium dodecyl sulfate (0.0125%, wt/vol) was included in the liquid medium used for growth and expression after the mutagenesis with ethyl methane sulfonate. This ensured that surface exclusion-deficient mutants would not

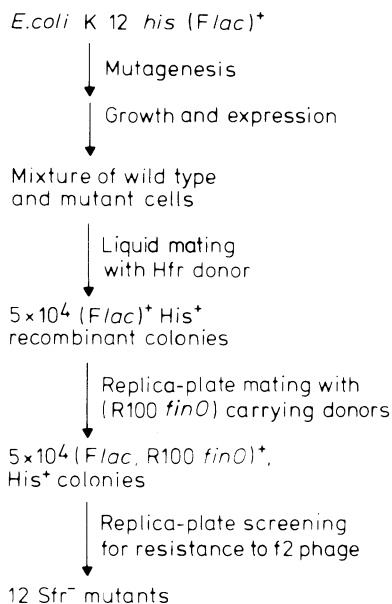


FIG. 1. Protocol for isolating mutants.

be lost by acting as recipients to wild-type transfer-proficient cells in the mixed culture (17).

(ii) Immediately after mutagenesis, 50 subcultures were established, and 1,000 colonies from each were later subjected to the analysis. Only one mutant was retained from any one subculture; therefore, many or all of the mutants tested carried independent mutations.

(iii) Two independent *finO* mutants of R100 (R100-1 and UCR105) were separately introduced into the colonies being tested to ensure that the inability to synthesize F pili was reproducible.

Once putative mutant colonies had been identified, their parental cells, which had not yet been exposed to the R100 *finO* plasmid, were purified by single-colony isolation and analyzed further. Twelve mutants were isolated. All twelve carried chromosomal *sfr* (sex factor regulation) mutations, because the mutant effects were still manifested after curing of *Flac* with acridine orange and the reintroduction of an unmutagenized *Flac*. No mutants were isolated with mutations on the F factor, and it remains to be seen whether the F factor can be mutated to *finO*⁺. We calculate that the 12 *sfr* mutants were isolated from an original 2×10^6 survivors of mutagenesis (5×10^4 multiplied by the 40-fold enrichment).

F factor expression in Sfr⁻ mutants. The F factor is known to encode proteins which result in plasmid DNA replication and segregation, plasmid incompatibility, inhibition of female-

specific phage multiplication, F pilus synthesis, DNA transfer, and surface exclusion (for review, see ref. 3). The cistrons responsible for these phenotypes map in three discrete regions of the F genome which are not known to share any common regulation. DNA replication and segregation cistrons map close to incompatibility and to sensitivity to curing by acridine orange. Because no preferential loss of plasmids was observed with the *sfr* mutants, and because *Flac* was easily curable from these mutants with acridine orange, the Sfr⁺ phenotype is not needed for those functions. Incompatibility has not been tested.

Replica plating revealed that all but one of the *sfr* mutants (that carrying the *sfrB1* mutation) were resistant to f2 bacteriophage whether they carried *Flac*, *Fgal*, or R100*drd*. Quantitative data with mutants carrying *Flac* are presented in Table 2 for the phenomena of DNA transfer, surface exclusion, and inhibition of female-specific phage multiplication. The results with *Fgal* or R100*drd* were comparable, except that R100*drd* does not affect female-specific phage development. The phenotypes of the mu-

TABLE 2. Phenotype of *sfrA* and *sfrB* cells carrying *Flac*

Group	Mutation	Transfer efficiency (%) ^a	Surface exclusion index ^b	Plating efficiency of ϕ_{II} (%) ^c
I	<i>sfrA4</i>	3.7	3.8	4.8
	<i>sfrA7</i>	3.2	3.5	5.3
	<i>sfrA10</i>	2.8	2.6	2.7
II	<i>sfrA8</i>	0.3	12.01	≤0.04
	<i>sfrA9</i>	0.1	9.0	≤0.04
	<i>sfrA5</i>	0.06	19.5	0.09
III	<i>sfrB1</i>	17.0	6.4	≤0.04
	<i>sfrB14</i>	8.5	10.5	≤0.04
	<i>sfrB2</i>	2.0	7.1	0.01
	<i>sfrB13</i>	1.0	3.5	0.1
IV	<i>sfrB3</i>	7.1	5.2	32.3 ^c
	<i>sfrB11</i>	0.02	12.6	50.8 ^c
WT ^d	<i>sfr</i> ⁺	100.0	390	0.5

^a Each value indicates the number of transconjugant cells which have acquired the *Flac* plasmid when expressed per 100 donor cells introduced to the mating mixture and after normalization for the value with the *sfr*⁺ control.

^b Each value indicates the ratio between the number of recombinant transconjugants obtained with F recipients and the number obtained with the F-prime-carrying test strain.

^c Obtained by normalization to a value of 100 for the *sfr*⁺ F control strain.

^d WT, Wild type.

^e Small, turbid plaques.

tants were not identical. Although only two mutated cistons, *sfrA* and *sfrB*, could be distinguished genetically (see below), mutants affecting each ciston fell into two groups (Table 2). Group I contained three *sfrA* mutants, all of which reduced DNA transfer ability and surface exclusion. Furthermore, ϕ_{II} bacteriophage plaqued slightly more efficiently on these mutants than on the parental *sfr*⁺ strain. Group II mutants were significantly poorer at DNA transfer but better at surface exclusion than were group I mutants. Furthermore, ϕ_{II} plaqued even less efficiently on these mutants than on the wild-type strain. Group III and IV mutants varied in their DNA transfer efficiency from 0.02 to 17.0% of the value with the *sfr*⁺ parent. All were moderately defective in surface exclusion, and group IV mutants were very efficient at plaquing ϕ_{II} bacteriophages. Surprisingly, neither the group I nor the group IV mutants were any better than the *sfr*⁺ control at plaquing T7, another female-specific bacteriophage. Despite their heterogeneity, two generalizations are possible. (i) All the *sfr* mutants are somewhat leaky, since residual DNA transfer and residual surface exclusion were detected. This may indicate that the mutations do not totally inactivate the corresponding gene products or that expression of *tra* functions is not totally dependent on function of these gene products. (ii) All the mutants affect the two independent properties of the *tra* operon (F pilus synthesis and surface exclusion) for which selection was imposed.

Genetic mapping of *sfr* mutations. We know of no technique which could be used for direct selection for the *Sfr*⁺ phenotype. Therefore, we used resistance to male-specific bacteriophages of cells carrying an F prime to screen for linkage of the *sfr* mutations to known cistrons. F⁻ cells carrying *sfr* mutations were used as recipients in conjugation with various Hfr donor strains and recombinants which were either His⁺, Trp⁺, Lac⁺, Gal⁺, or Mal⁺ were selected. *Flac* was then introduced into the recombinants by conjugation to test whether they had inherited *sfr*⁺ from the Hfr donor cells. As a result, we could assign six mutations to a locus, *sfrA*, mapping between the points of origin of Hfr's Hayes and $\chi 313$ and the six others to a locus mapping between the points of origin of KL228 and P72 (Fig. 2). P1 transduction was then used to refine these map positions. The *sfrA* mutations are cotransducible with *thr* into strain M1986 at a frequency of 0.95 and with *ara* at a frequency of 0.34. *sfrB* mutations are cotransducible with *metE* into strain M3960 at a frequency of 0.3 and with *cya* at a frequency of 0.01. Thus, the *sfrA* locus maps at 0/100, and

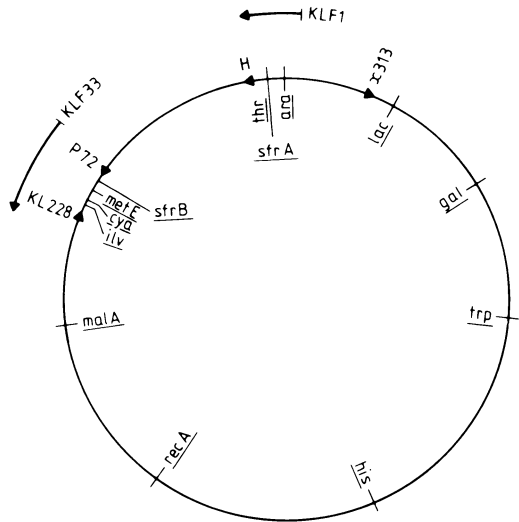


FIG. 2. Map of the *E. coli* chromosome according to Bachmann et al. (8). The points of origin of the Hfr strains KL228, P72, H and $\chi 313$ are indicated by arrowheads. The chromosomal DNA carried by the F primes KLF33 and KLF1 are shown by the lines outside the circle; their origins of transfer are indicated by arrowheads. The positions of *sfrA* and *sfrB* are based on the results given in the text.

the *sfrB* locus maps at 84 on the current *E. coli* K-12 map (8). These results also render the possibility unlikely that the *Sfr*⁻ phenotype is due to multiple mutations, since no difficulties were encountered with transduction. Since strain M1986 carries the Su_{II}⁺ amber-suppressor gene, the *sfrA* mutations are unlikely to be amber mutations.

sfrA is highly cotransducible with *thr*, and we were unable to decide the relative positions of *sfrA* and *thr* from three-point transductional mapping experiments. In contrast, we were able to define the map order of *sfrB* and *metE*. The cotransduction frequency of *ilv* and *metE* was 0.13, whereas cotransduction of *ilv* and *sfrB* was undetectable (<0.0001). Thus, *sfrB* probably maps distal to *metE*, relative to *ilv*. Three-point transductional analysis using the *cya* marker (Table 3) demonstrated that the order is indeed *ilv cya metE sfrA* (Fig. 2).

The *sfrA* and *sfrB* mutations are recessive because introduction of the F primes KLF1 into the former and KLF33 into the latter restored the cells to the *Sfr*⁺ phenotype; these cells transferred the F primes efficiently and were sensitive to male-specific bacteriophages. Occasional colonies contained homozygote cells in which the *sfr* mutations had moved to the F primes. We purified four such colonies carrying the mutant F primes pBE501 (KLF1, *sfrA4*), pBE502

TABLE 3. *Fine mapping of *sfrB* with P1 transduction and three-point linkage analysis*^a

Selected marker	Unselected markers	Frequency of recombinants	No. of crossovers required	
			<i>cya</i> - <i>metE</i> - <i>sfrB</i>	<i>cya</i> - <i>sfrB</i> - <i>metE</i>
<i>cya</i> ⁺	<i>metE sfrB</i> ⁺	0.84	2	2
<i>cya</i> ⁺	<i>metE</i> ⁺ <i>sfrB</i> ⁺	0.15	2	4
<i>cya</i> ⁺	<i>metE</i> ⁺ <i>sfrB</i>	0.01	2	2
<i>cya</i> ⁺	<i>metE sfrB</i>	≤0.0008	4	2

^a P1 lysates grown on all six JC3272 derivatives carrying *sfrB* mutations (*cya*⁺ *metE*⁺ *sfrB*) were used to transduce M3960 (*cya metE sfr*⁺) with selection for Cya⁺ or Met⁺ (data not shown) transductants. A total of 200 were tested from each transduction. Since no significant differences were found among the six *sfrB* mutations, the data for all 1,200 transductants have been summarized here. The data with Met⁺ transductants were also inconsistent with the order *cya sfrB metE*, since the putative four-crossover class was the most frequent.

(KLF1, *sfrA10*), pBE503 (KLF33, *sfrB13*), and pBE504 (KLF33, *sfrB14*). When introduced into *sfr*⁺ hosts by (inefficient) conjugation, these mutant F primes stimulated F pilus synthesis and DNA transfer. When introduced back into cells carrying the *rec*⁺ or *recA56* alleles plus each of the corresponding *sfr* mutations, no complementation was observed and F pilus synthesis and DNA transfer were both inhibited. Thus, all the *sfrA* mutations belong to one cistron, and all the *sfrB* mutations belong to a second cistron. This conclusion theoretically could be artefactual, due to pBE501 through pBE504 arising by deletion within KLF1 or KLF33 rather than by homozygization. However, *sfr*⁺ recombinants were detected in a *rec*⁺ strain between *sfrA4* on pBE501 and the other *sfrA* mutations on the chromosome except for *sfrA7* and *sfrA10*. Similar recombinants were detected between *sfrB13* on pBE503 and all the other *sfrB* mutations on the chromosome. Thus, this possibility is unlikely. The experiments described below could therefore safely be performed with representative mutations affecting the *sfrA* and *sfrB* cistrons rather than with all 12 mutants.

Other conjugative plasmids. Plasmids belonging to 15 different incompatibility groups have been tested for the ability to transfer themselves from cells carrying *sfrA8* or *sfrB13* mutations. The plasmids could be assigned to four groups, based on their response (Table 4). The results show that *sfrA* and *sfrB* are specific, since most plasmids other than the F-like plasmids did not require function of either the *sfrA* or *sfrB* cistrons. Furthermore, *sfrA* and *sfrB* have different specificities, since some plasmids

required function of only one of the two and others required function only of the second. Most of the F-like plasmids tested were dependent on the function of both cistrons. However, ColV2 needed only *sfrA*, and R1*drd-19* needed only *sfrB*. Furthermore, the C and S group plasmids, which are not known to share any DNA homology with F, were also dependent on *sfrB* function.

Synthesis of extracellular organelles. Derivatives of JC3272 carrying *Flac* plus an *sfrA* or *sfrB* mutation were examined by electron microscopy for their ability to synthesize F pili (Table 5). Both *sfrA4* and *sfrA5* mutations abolished detectable F pilus synthesis. However, 2/105 *sfrB11* cells and 21/100 *sfrB13* cells possessed F pili. This number is lower than for *sfr*⁺ cells and indicates a leaky inhibition of F pilus synthesis. The results with *sfrB13* indicate more F pilus synthesis than had been anticipated from the low efficiency of DNA transfer by these cells (Table 2) and from the fact that they are resistant to male-specific bacteriophages. We have no explanation for this discrepancy.

JC3272 cells synthesize common type 1 pili, and some of these cells synthesize flagella. The *sfr* mutations had no effect on type 1 pilus

TABLE 4. *Transfer from mutant strains of different conjugative plasmids*

Group	Dependent on:		Plasmids (compatibility groups)
	<i>sfrA</i>	<i>sfrB</i>	
A	Yes	Yes	F (FI), ColVB <i>trp</i> (FI), R100 <i>drd</i> (FII), R538-10 ⁺ (FII), ColB4 (FIII), R124 (FIV)
B	Yes	No	ColV2 (FI)
C	No	Yes	R1 <i>drd-19</i> (FII), R55 (C), R40a (C), R478 (S), R479b-1 (S)
D	No	No	Ral (A), R16 (B), R27 (H), R64 <i>drd-11</i> (Ia), R446b (M), N3 (N), RP4 (P), R ₁₈ (T), S-a (W)

TABLE 5. *F pilus production by *sfrA* and *sfrB* mutants*^a

Mutation	F pili/cell	Fraction of F-piliated cells
<i>sfr</i> ⁺	1.06	0.76
<i>sfrA4</i>	≤0.01	≤0.01
<i>sfrA5</i>	≤0.01	≤0.01
<i>sfrB11</i>	0.02	0.02
<i>sfrB13</i>	0.25	0.21

^a For each of the five strains (JC3272 carrying *Flac* plus the mutation indicated), 100 or more cells were examined by electron microscopy. When F pili were seen, they usually carried numerous M12 bacteriophages.

synthesis, but fewer of the *sfrB* cells possessed flagella than the *sfr*⁺ cells or than the *sfrA* mutants. Furthermore, the *sfr*⁺ and all six *sfrA* mutants exhibited normal motility on swarming plates, whereas five of the six *sfrB* mutants were less motile. The sixth mutation, *sfrB1*, is quite leaky (Table 2). It was not possible to extend this analysis with these strains, because only some JC3272 cells synthesize flagella and others do not. Furthermore, JC3272 is resistant to χ bacteriophage, which requires functional flagella for infection of the cell. M3960 when supplemented with cyclic AMP synthesizes normal numbers of flagella and is sensitive to χ bacteriophage. Furthermore, the *sfrB* mutations had been introduced into M3960 by P1 transduction. M3960 and derivatives carrying the *sfrB11* and *sfrB13* mutations were tested. Both mutants were resistant to χ bacteriophages and did not swarm normally on swarming plates. The mutants were restored to the wild-type phenotype by introducing the *sfrB*⁺ F-prime KLF33. Electron microscopic analysis of the mutants revealed that only a few of the mutant cells synthesized flagella (Table 6). Most of the few flagella synthesized did not bind χ bacteriophages and were short and rigid rather than long and sinuous. These were scored as defective flagella. We conclude that *sfrB* is needed for efficient synthesis of functional flagella.

Response of Sfr⁻ mutants to bacteriophages and antibiotics. The effect of the *sfrB* mutations on flagella synthesis suggested that these mutations might influence other cell surface functions. We therefore tested the sensitivity of the *sfr* strains to a series of bacteriophages which use various components of the cell surface as receptors for absorption and DNA injection (15, 16). Spot tests were used to screen various bacteriophages, followed by quantitative titration of plaquing efficiency in those cases which showed differences between *sfr*⁺ and *sfr* mutants. The results (Table 7) show that the *sfrA* mutations had no detectable effects, whereas the *sfrB* mutations did. The effects of the *sfrB* mutations could be reversed by introducing the *sfrB*⁺ F-prime KLF33 into the *sfrB* mutants.

The pattern of altered bacteriophage sensitivity suggests that the *sfrB* mutations affect the

TABLE 7. Response to bacteriophages

Group of bacteriophages	Members	Response of: ^a		
		<i>sfr</i> ⁺	<i>sfrA</i>	<i>sfrB</i>
a	U3	S	S	R
	C21	R	R	S
b	T1, T2, T4, T7, BF23	S	S	S
c	T6, ϕ 12, K3, λ	S	S	NT

^a S, Sensitive; R, resistant; NT, not tested because the two available *sfr*⁺ parental strains were themselves resistant. *sfrA4* and *sfrA5* were used as representative *sfrA* mutations, whereas *sfrB11* and *sfrB13* were used as representative *sfrB* mutations.

lipopolysaccharide (LPS) of the outer membrane. Bacteriophages C21 and U3 both use the LPS as a receptor for absorption (23, 28). Normally, *E. coli* K-12 is sensitive to U3 and resistant to C21. *E. coli* B shows the opposite pattern (21, 28), and the difference has been ascribed to different LPS compositions (9, 23, 28). Because some *gal* mutations in *E. coli* K-12 lead to LPS changes which result in resistance to U3 and sensitivity to C21 (23, 28), the possibility existed that the response of the *sfrB* mutants was an artefact due to the *gal* mutation present in JC3272. However, these Sfr⁻ strains showed the same effects even when they carried *Fgal* and when the growth medium was supplemented with galactose.

Cell envelope changes are sometimes also associated with conjugation deficiency or altered sensitivity to antibiotics. The Sfr⁻ mutants yielded as many recombinants after conjugation with Hfr donors as did their Sfr⁺ parent. However, the *sfrB* mutants did differ from both *sfr*⁺ and *sfrA* cells with regard to antibiotics. All *sfrB* mutations resulted in significantly greater sensitivity to chloramphenicol (10 μ g/ml) and novobiocin (30 μ g/ml). Lipopolysaccharide mutants which are resistant to bacteriophage U3, sensitive to phage C21, and supersensitive to novobiocin have been described (16). Thus, *sfrB* mutations seem to affect the lipopolysaccharide composition.

DISCUSSION

The *sfrA* and *sfrB* cistrons defined here are apparently distinct from previously described *E. coli* K-12 cistrons. However, similar mutants which may carry *sfrA* or *sfrB* mutations have recently been isolated (22; P. Silverman and M. Malamy, personal communications). The gene products of *sfrA* and *sfrB* are needed for efficient expression of the conjugation proteins of most F-like conjugative plasmids. Their normal role for *E. coli* cells is not clear.

All the mutants analyzed here are leaky. This

TABLE 6. Flagella synthesis by *sfrB* mutants

Mutation	Fraction of cells with:		
	Normal flagella	Defective flagella	No flagella
<i>sfr</i> ⁺	0.67	0.14	0.19
<i>sfrB11</i>	≤ 0.01	0.11	0.89
<i>sfrB13</i>	0.05	0.17	0.78

could represent residual function of a partially mutated gene product or the possibility that the functions analyzed are not totally dependent on these gene products. In the absence of this information, it is not clear whether the *sfrA* and *sfrB* gene products play an important and general role in cell growth and metabolism. However, expression of F pilus synthesis, DNA transfer, and surface exclusion by F *tra* cistrons is clearly dependent on full function of both the *sfrA* and *sfrB* cistrons. Furthermore, inhibition of ϕ_{11} bacteriophage development by F *pif* cistrons, as well as flagellar function and other properties (sensitivity to bacteriophages C21 and U3 and to antibiotics), are all at least partially dependent on the *sfrB* gene product. Many of these phenotypes reflect the function of the cell surface, and the *sfrB* gene product may play an important role in the regulation of synthesis or of the function of the *E. coli* cell surface.

At what level are the *sfr* gene products required? The effects of the *sfr* mutations on F factor genes are easiest to interpret. Because both F pilus synthesis and surface exclusion are impaired and because different *tra* cistrons independently encode these two cell envelope-associated functions, the *sfr* mutations apparently exert a pleiotropic effect on *tra* cistrons. Such an effect could be at the transcriptional level or at posttranscriptional levels. The *tra* cistrons affected are located in the *tra* operon, the transcription of which is dependent on the function of *traJ* (29). Thus, the *sfrA* and *sfrB* gene products could be involved either in transcription of the *tra* operon or in transcription of *traJ*. However, the data presented here do allow one possible transcriptional mechanism to be excluded. These mutants do not synthesize a novel repressor protein similar to the *finO* protein, since the mutations were all recessive and a repressor would have been dominant. The possibility that the *sfrA* and *sfrB* gene products are needed at a posttranscriptional level rather than for transcription should not be discounted. Since the *sfrB* mutations resulted in several cell envelope-associated changes, their phenotype might in fact be due to indirect effects on function within the cell envelope of proteins that are synthesized at a normal rate. In fact, the observation that the quantitative effects of the *sfrA* mutations on DNA transfer and surface exclusion were not correlated may reflect just such an indirect effect.

Despite the current uncertainties about the normal roles of the *sfrA* and *sfrB* gene products, the existence and properties of these mutants do demonstrate that the host cell plays a specific role in the expression of conjugation cistrons of

the F sex factor. Further analysis of these mutants may, therefore, shed light both on the basis of conjugation and the mechanisms of cellular function.

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